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Short Communication

Toxicological and Growth Regulatory Effects of Acetone Extract Oils of Indigenous Medicinal Plants Against a Stored Grain Pest, *Cryptolestes ferrugineus* (Stephens) (Coleoptera: Cucujidae)

Muhammad Sagheer,[1] Mansoor-ul-Hasan,[1]* Mazhar Hussain Ranjha[1], Umair Sagheer,[2] Shahzad Saleem,[3] Qurban Ali,[1] Kazam Ali[1] and Abdul Majid[1]

[1]*Department of Entomology, University of Agriculture, Faisalabad, Pakistan*
[2]*District Food Controller, Punjab Food Department, Shaikhupura, Pakistan*
[3]*Department of Biosciences, COMSATS Institute of Information Technology, Sahiwal*

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Authors' Contribution

SS and QA collected experimental material. US, KA and AM performed the experiments. MHR wrote the article and he alongwith MS and M supervised the work.

Key words

Plant essential oil, contact toxicity, growth inhibition, *Cryptolestes ferrugineus*

ABSTRACT

Essential oils of *Melia azedarach*, *Linium usitatissium*, *Ferula narthex*, *Sasurrea costus*, *Viola odorata* and *Achyranthus aspera* were tested for their contact toxicity and growth regulatory effects against *Cryptolestes ferrugineus* (Stephens). The highest mortality (9.70%) was observed with 15% *M. azedarach* followed by *F. narthex*, *S. costus*, *A. aspera L. usitatissium* and *V. odorata*. The increase in exposure time was also directly related with mortality. Larval emergence and pupation were minimum (21.66%, 40.01%, respectively) in the presence of *M. azedarach* and maximum 89.84% and 90.14%, respectively in control. The overall results suggest acetone extract which may contain essential oils have considerable potential to protect stored grain by killing or inhibiting the growth of insect pests and might be used as grain protectant.

The *Cryptolestes ferrugineus* (Stephens) (Coleoptera: Cucujidae) is a cosmopolitan insect pest of stored products, especially stored grain (White, 1995). *Cryptolestes* species are incapable of damaging whole kernels in good condition but they may be associated with primary invaders such as *Sitophilus* spp. and *Rhyzopertha dominica* (F.). *C. ferrugineus* often occurs in mixed infestations with *Tribolium castaneum* (Suresh *et al*., 2001).

The pesticides are frequently used to prevent the post harvest grain losses. These chemicals are most cost-effective to prevent or control the insect infestations of stored products (Hidalgo *et al*., 1998). Among them, synthetic insecticides and fumigation are the main methods to control stored-product insect pest. However, recently documentation of some serious issues *i.e.*, development of insect pest resistance, human health hazards, environmental pollution and effect on non target organisms arouse the need for new technologies to manage the stored-product insect pests (Fields *et al.*, 2001; Isman, 2006). Many medicinal and ornamental plants have the natural insecticidal properties. Plant essential oil treatment is inexpensive and convenient method to protect the grains from insect pest infestation on small farms and households. The oil does not affect the germination rate and flavors of grains (Rajashekar *et al*., 2010). Thus, there is an urgent need to develop safe alternatives to conventional insecticides and fumigants for the protection of grain products against insect infestations. There are increasing efforts to understand indigenous pest control strategies, with a view to revive and modernize their use (Shaaya *et al*., 1997). Plant products have played an important part in traditional methods of protection against insect infestation. Plant derived chemicals such as rotenone, pyrethrum, and nicotine, used in have been the West-africa, Indo-Pakistan subcontinent and other Asian countries (Phillipson, 2001).

Plant extracts contain compounds that show antifeedant, repellent, ovicidal and toxic effects against insects (Nawrot and Harmatha, 1994; Isman, 2006). Many plants in Pakistan have been successfully used as repellents against insect pests of stored grains (Jillani *et al*., 1993). Saleem *et al*. (2014) evaluated insecticidal activity of essential oils of indigenous plants from Pakistan and found strong fumigant effect against *T. castaneum*, *T. granarium* and *C. ferrugineus*. The aim of present study was to determine the insecticidal effects (toxicity and growth inhibitory effects) of the essential oil of indigenous medicinal plants such as *Melia azedarach*, *Linium usitatissium*, *Ferula narthex*, *Sasurrea costus*, *Viola odorata*, and *Achyranthus aspera* against stored

* Corresponding author: mansoorsahi2000@yahoo.com
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grain pest *C. ferrugineus*.

Materials and methods

Samples of adults of *C. ferrugineus* were collected from the flour mills and grain market located in district Faisalabad, Pakistan. The insect culture was maintained on wheat flour in sterilized jars that were placed in the incubator at 30±2°C and 60% R.H. to obtain the homogenous (F_1) population. The culture medium was wheat flour sterilized at 60°C for 60-90 min. Fifty beetles were liberated in 250 g of wheat flour placed in each jar of 9.5cm diameter. The jars were covered with muslin cloth, tied with rubber bands to avoid the escape of beetles. After five days, the adults were sieved out and the flour with eggs of insects was again shifted to the jars. The jars were placed in incubator at 30±2°C and 60% R.H (Model MIR-254, SANYO) for rearing of homogenous population.

For preparation of acetone extracted oils, the fresh leaves of *M. azedarach, V. odorata*, and *A. aspera* were collected from the various fields and gardens in the Faisalabad, washed in water, dried in shade and ground in electrical grinder (Machine No. 20069, Pascall Engineering Co. Ltd.) to form powder. The fresh seeds of *F. narthex, S. costus* and *L. usitatissium* were obtained from herbal shop of the local market, washed and then soaked in water for 24 h. The extraction of most of the organic molecules including oils was done using Soxhlet's extraction apparatus by running 50 g of powder in 250 ml acetone according to procedure described by Valladares *et al.* (1997). Sufficient amount of plant extract in acetone were kept in clean and air tight glass vials. The samples were stored in the refrigerator at 4ºC before use.

For determination of contact toxicity of oils experiments were carried out in 80 mm plastic Petri dish and Whatman filter No. 1 paper was used for bioassay. Different concentrations of acetone extract oils (5, 10 and 15%) were applied on the filter paper and then the filter paper was allowed to dry for 10 min. Three replications were used for each concentration and filter paper was treated with acetone alone as a control. Twenty, one week old adults of *C. ferrugineus* were introduced in the Petri dish which was covered by the lid. Mortality of test insects was recorded 24, 48 and 72 h after the application.

For determining growth inhibitory effects of oil experiments were carried out in small plastic vials. Ten ml of different concentrations of acetone extracted oils (5, 10 and 15%) were applied on flour and was allowed to dry in air for 10 min. Three replications were used for each concentration and flour was treated with acetone alone as control. Mixed population of twenty adults (male and female) of *C. ferrugineus* was introduced in vials for egg lying which were covered with meshed cloth. Data for larval emergence and % pupation was recorded after every two days in F_1 generation.

The data was analyzed under CRD for analysis of variance and the means of the treatments were separated using Tukey-HSD test. The Statistica software (Stat Soft, 8.0) was used for statistical analysis of the recorded data.

Results and discussion

Table I shows different concentrations of acetone extracted oils of different plants administered for a period of 24, 48 and 72 h on larval emergence, pupation and mortality of adults of *C. ferragineus*, respectively. The highest adult mortality (9.70%) was observed in the case of *M. azedarach* 15% concentration, followed by *F. narthex, S. costus, A. aspera, L. usitatissium* and *V. odorata.*

The contact mortality against adults of *C. ferrugineus* with respect to different exposure period revealed that there was direct relation between mortality and exposure time (Table II). The highest mortality (9.34%) was recorded with *M. azedarach* after 72 h of treatment application followed by *F. narthex* and *S. costus*, while *V. odorata* extracted oil gave the minimum mortality. Same trend was noticed after 48 h and 24 h of treatment application.

Table I also showed that larval emergence and pupation was minimum in *M. azedarach* at 15% concentration and the highest larval emergence against *V. odorata* and pupation against *L. usitatissium* at 5% concentration.

Effectiveness of *M. azedarach* as bio-pesticide against *C. ferrugineus* was also documented by Li (2008). Previous studies also reported the supporting findings where diversified growth inhibitory effect of plant extracts (*Melia volkensii*, *Melia azedarach*) were confirmed against cabbage looper and rice leaf folder, respectively (Akhtar and Isman, 2004; Senthil, 2006). These results are also in agreement with the findings of Silva *et al.* (2012). Growth inhibition in *C. ferrugineus* caused by different plant extracts indicated that these extracts inhibited vitellogenesis, oviposition, egg hatching and larval mortality. Many phytochemicals inhibit vitellogensis in insects (Dorn, 1986, Sayah *et al.*, 1996). Our results regarding *M. azedarach* against *C. ferrugineus* were in consistence with the findings of Xie *et al.* (1995) who confirmed that the azadirachtin was largely responsible for toxic actions of neem on the stored-product insect pests. Our results are contrary to the findings of Athanassiou *et al.* (2005), who reported that the azadirachtin based insecticides Neemazal was not very effective against stored grain insect pest. The

Table I.- Effect of different concentrations of plant acetone extracted oils on larval emergence, pupation and mortality of adult beetles of *C. ferrugineus* adults.

		Control	Concentrations of plant acetone extracted oils		
			5%	10%	15%
Adult mortality (%)	*M. azedarach*		3.76±0.49 b	6.35±0.78 a	9.70±1.57 a
	F. narthex		2.64±0.37 b	5.61±0.92 a	9.33±1.41 a
	S. costus		1.90 ±0.58 b	3.75±0.49 b	7.84±1.26 a
	A. aspera		2.27±0.49 b	3.76±0.74 ab	5.61±0.93 a
	V. odorata		0.78±0.56 b	1.56±0.58 ab	3.38±1.17 a
	L. usitatissium		1.53±0.59 b	2.27±0.49 b	4.49±0.58 a
Larval emergence (%)	*M. azedarach*	65.33±0.88d	43.00±0.58c	34.33±1.76b	21.66±0.88a
	F. narthex	78.67±2.03d	55.00±0.58c	46.66±0.03b	32.33±0.89a
	S. costus	81.67±0.89d	65.33±0.33c	58.00±0.57b	46.66±1.45a
	A. aspera	82.67±0.89d	72.67±0.89c	62.00±0.57b	51.33±1.45a
	V. odorata	81.00 ±0.57d	75.00±1.14c	64.00±0.57b	56.33±0.66a
	L. usitatissium	89.84±2.45d	72.89±1.23 c	57.37±1.75 b	40.01±0.51a
Pupation (%)	*M. azedarach*	89.84±2.45b	72.89±1.23c	57.37±1.75b	40.01±0.51a
	F. narthex	91.12 ±0.50c	77.01±2.79b	64.46±2.27a	57.67±1.42a
	S. costus	93.04±1.12c	82.64±2.24b	80.50±2.30b	73.32±3.07a
	A. aspera	90.61±0.81c	81.31±1.17b	76.58±2.81b	63.16±2.18a
	V. odorata	91.12±0.49c	85.82±1.43a	81.19±0.91a	79.84±1.43a
	L. usitatissium	94.25 ±1.05 c	90.14 ±1.44 b	84.37± 0.14ab	81.09 ±1.36 a

Table II.- Effect of plant essential oils on mortality of *C. ferrugineus* adults at different exposure periods.

Exposure periods (h)	*M. azedarach*	*F. narthex*	*S. costus*	*A. aspera*	*V. odorata*	*L. usitatissium*
24	4.12±0.56b	3.38±0.87b	3.01±0.58 b	1.89±0.74b	0.78±0.82b	2.27±0.74 a
48	6.35±0.78 a	5.98±0.87 a	4.49±1.38ab	4.13±0.56a	1.89±0.0.58ab	2.64±0.67 a
72	9.34±1.71 a	8.21±1.68 a	5.98±1.30 a	5.61±0.92 a	3.01±1.24 a	3.38±0.67 a

contrast results may be due to differences in material and methods.

The results of the current and earlier research activities suggest that the botanical extracts are promising larvicides against *C. ferrugineus.* Moreover, these results could be useful in the search of newer, more selective and biodegradable pesticide.

Conclusion

The result revealed that mortality was increased with the increase in the concentration of essential oil and exposure time, but the essential oils of *M. azadirach, F. narthex, S. costus* and *A. aspera* showed more potential compared to essential oil of *V. odorata* and *L. usitatissium*.

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